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CELL CHANGES IN THE ALVEOLI OF A CARCINOMA OF THE MAMMA.

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Plates XLVI to XLVIII.

THE literature upon the pathological new growths of the human body scarcely mentions amitosis as a method of cell increase. It occurred to me to treat in a comparative way the two methods of cell division, mitosis and amitosis, as they are found in neoplastic material.

It is not intended to make this paper conclusive, but to make it the first of a series of case studies from the results of which a general conclusion may be deduced as to the relative importance of indirect and direct cell division in these new growths.

I am indebted to Dr. M. T. Sudler for the following history: "In the first week of October, 1907, she fell, bumping the breast against a hard object. No further notice was taken of the incident, however, until the latter part of November, when she noticed a hard, thin, round lump in the right breast about the size of a silver dollar. It was then freely movable. About the 25th of December she noticed a tingling in the right arm, and on January 1, 1908, the arm began to ache. The original mass increased rapidly in size until the whole breast was involved. Its rate of growth seemed intermittent, some weeks being much more rapid than others. Upon examination the entire breast was found to be occupied by a hard, firm mass, which was intimately incorporated in the connective tissue of

the breast. The nipple was somewhat retracted but not completely. The mass was movable over the muscle. The axillary nodes were enlarged and hard. On February 5 the breast, together with the axillary lymph nodes, was removed. On March 13 a recurrence in the left breast was discovered, which was removed on March 20. Twelve days later metastatic nodules in the neck were removed. The patient died soon afterwards from an apparently general carcinomatous involvement of the body. From the foregoing history it is plain that this was an extreme type of the most rapidly growing and malignant of tumors."

This material was not prepared especially for fine cytological work because the discovery of its excellence for investigation was not made until after its fixation. It was fixed in ten-per-cent formalin and remained in a weaker solution until used. I found the cell nests somewhat shrunken away from the surrounding stroma and the chromatin either so swollen or so poorly preserved that I was unable to make out details in regard to the chromosomes. The fixation otherwise was good. The tissue was mounted in paraffin and cut in sections eight micra thick. Sections were stained in hæmatoxylin for twelve hours after being in a mordant of iron-alum for twelve hours. They were then decolorized in the iron-alum solution to the desired extent.

The cells studied appeared in variously shaped pockets or alveoli surrounded by dense white fibrous connective tissue. This connective tissue stroma was penetrated more or less by columns of tumor cells, one to three cells in diameter. In short, this specimen in parts conformed to the scirrhus while in others it was more of the medullary type of carcinoma. In this paper I will confine my study to the cell nests or alveoli.

In the first four figures I chose a typical field from widely separated parts of the tumor for each drawing, and drew all the structures appearing in that field. I did this to show the similarity of the cell content of the tumor mass throughout. The normal resting cell, with its variation in size, shape and chromatin content, composed the greater number of cells of each group. It was considered the normal resting cell because of its uniform staining quality, its maintenance of cell and nuclear wall, its content of regularly outlined chromatin nucleoli within a single nucleus. Marked variation in any of these

characteristics was considered a cell change having as its ultimate end either the production of new cells or degeneration.

Among the resting cells were others which could be easily identified as being in the process of indirect division. Although no detail of the chromosomes could be made out, yet the increased amount of chromatin over the resting cell, its affinity for the stain, the irregular outline of the chromosomes, the absence of the nuclear membrane and the shape and position of the chromatin masses identical with those of the prophase, metaphase, anaphase, and telophase leave no doubt as to the identity of such cells. Equally distributed among the resting cells were other cells which were clearly abnormal, owing to their unusual size and their content of two or more nuclei or clefts of greater or less depth which either separated or tended to separate the single nucleus into two or more distinct segments. These separated segments had all the characteristics of the nucleus of the resting cell. I have considered such cells as in the process of direct cell division, although I have been unable to find a single cell showing distinct separation of the cytoplasm. Adami thus describes amitosis: "The nucleus divides without any apparent preliminary rearrangement of its structure. It becomes elongated, then dumb-bell shape (the process can be followed in the amœba), the connecting neck becomes broken across and the two daughter nuclei pass apart, their separation being in some cases followed by division of the cell body while in others this further division is wanting and the binucleate cell is produced." Patterson, in "Amitosis in the Pigeon's Egg," says: "In most cases the nucleus elongates in the direction transverse to the plane of the future division, after which a constriction in the nuclear membrane appears about the entire circumference of the nucleus. This constriction continues to deepen until a complete division is effected. A modified form of this type is found in those cases in which the constriction proceeds from one side."

Although the process in this material differs somewhat, I think, from that described by Adami and Patterson for other material, the result is the same. Therefore I feel safe in assuming that cells with the nucleus wholly or in various degrees of separation are in the process of amitosis. Ziegler and Vom Rath consider such cells as already in the process of degeneration. If so, it will not be out of place to consider along with these division figures structures that appear throughout

this material and which are plainly cells in varying degrees of degeneration, as evidenced by changes in the chromatin, cytoplasm and cell wall, and note if there is any similarity between them.

Figures 5, 6, 7 and 8 are used to further illustrate these three cell changes as they appear in various parts of the tumor. In these drawings I omitted the greater number of the resting cells.

I will supplement this preliminary account by short descriptions of individual cells in the several plates with interpretations of their variation from the normal.

I have used the term "cell nests" to mean the group of cells within the alveoli; the term "chromatic cells" to mean those cells in that condition in the degeneration process just before the chromatin loses its staining power and when it has swollen so much and become so diffuse as to fill the entire nucleus as a more or less densely staining mass.

OBSERVATIONS.

The Cells of the Nests Change by Mitosis, Amitosis and Degeneration.

In showing the cell changes of these nests I will simply use a number of figures, describing each one with some amount of detail and inserting comments as observations suggest.

FIGURE 1 is drawn from cell nest 2. It includes three cells dividing directly, one dividing indirectly, two cells in the process of degeneration, and a number of resting cells.

The resting cell is smaller than the dividing cell, is of polyhedral shape, has a limiting membrane, and a large, well-marked nucleus. Its cytoplasm is denser than the karyoplasm and is of a reticular appearance. The nucleus has a well-marked nuclear wall and usually contains from one to three regularly round, deeply-stained chromatic bodies which I will call chromatin nucleoli. A directly dividing cell is of much larger size than the resting cell and usually a bit clearer, a condition suggesting that it has included a large amount of fluid preparatory to its division. The nucleus divides by the formation of a groove or cleft on one side. I have not seen a cell where the cleft appeared on the two sides. The cleft deepens and the nuclear substance pulls apart to form the regular outline of the daughter nuclei. The nuclear membrane seems to follow the deepening cleft so that as soon as the segments have separated the karyoplasm is enclosed by its wall.

In the cells shown I have drawn them as they appeared in outline in one plane, but included the chromatin through the depth of the cell. Each well-defined segment of the nucleus usually contains a chromatin nucleolus. In cell *A* the lower nuclear segment has a second cleft appearing upon its inner border. Cell *B* has two distinct nuclear segments, each one of which has a secondary groove appearing upon its upper surface. The upper segment shows no chromatin at all. Cell *C* shows two nuclear segments, each containing a chromatin nucleolus. Cell *D* shows a central constriction of its nucleus with the mother chromatic mass in the plane of constriction, but I do not say it is in the process of division.

Cell *E* is a representation of the typical indirect division figures found in this material. It is in the early anaphase. As I have already said, this material, being fixed in formalin with no particular care regarding its cytological preservation, shows no detail characteristics of the chromosomes. I have used in my counts only the well-marked division figures, from the close spireme stage on. In these indirect divisions the cells are larger than the resting cells, the nuclear membrane had disappeared, and about the chromatic figure the cytoplasm was clear. I was unable to make out the spindle or any of the details farther than shown.

Cells *F F* are two fragments of degenerated cells in each of which the chromatin has swollen and coalesced into one homogeneous, deeply staining mass.

FIGURE 2. Among the cells of this group there is not the noticeable difference in size usually seen between the dividing cells and the resting ones. Cell *A* shows an indirect division plainly. Cell *B* shows the nucleus being divided by a cleft passing in from its upper surface. Each segment contains a chromatin nucleolus. Cell *C*, an otherwise ordinary resting cell, contains in its cytoplasm, half way from the cell border to the nuclear membrane, a clear vesicle, regular in outline and with a definite limiting border indicating that it is filled with a substance differing from the cytoplasm.

FIGURE 3 shows no new structures, but attention is called to the regularity of the chromatin nucleoli. Cells *A* and *B* show the nuclei entirely segmented and each segment containing regular chromatin nucleoli, the lower segment of cell *B* containing two.

FIGURE 4 is a group of twelve tumor cells drawn from cell nest 1, a small nest of seventy-five cells containing six indirect and no direct divisions. In this group are shown four of the six indirect divisions. Cells *A* and *B* are noticeable for their large size, while cells *C* and *D* are of the same size as the resting cells. Cell *E* is a common-sized resting cell, containing one chromatin nucleolus but with a hollow clear vesicle bulging into the wall of its nucleus. Cell *F* is a common resting cell except that it has its chromatin diffused through the karyoplasm.

FIGURE 5. In this group and in those following I have not included all the cells that appeared in the field, but simply instructive ones and enough resting cells to show a comparison. The group was drawn from cell nest 3, of 253 cells, which included six indirect and five direct divisions. Cell *C* was in the fourth tier from the edge and on the peripheral side of the group. It was large, being .0125 mm. in its greatest diameter and showing a nucleus in two segments, each segment containing a regularly round chromatin nucleolus. The cell was noticeably clearer than the others. Cell *B* also has a bisegmented nucleus, but its chromatin was scattered in irregular masses through the karyoplasm. It was also large, being .01 mm. in its greatest diameter. The nucleus of cell *A* was in three segments, each part having a regularly round chromatin nucleolus, and beside this a certain amount of chromatin scattered in clumps through the karyoplasm. It was .0125 mm. in its greatest diameter. In all my cell measurements I used a micrometer scale which I have found by measurement to give the following dimensions: With a drawing board on a level with the object and at the same inclination as the microscope stage, using 1/12 oil-immersion objective and a 8× Huyghenian ocular, an object that fills the square of the scale is .0125 mm. in diameter; with the 1/6 objective it is .03 mm. in diameter, and with a 2/3 objective it is .15 mm. in diameter. Three-fourths of the square equals .0093 mm.; one-fourth of the square is equal to .0031 mm.; one-half of the square is equal to .0062 mm. Cell *D* is in the indirect division process. Cell *E* is a structure that is common among the tumor cells and I have concluded that it is a degeneration result, although it may occur anywhere in the cell nest among the otherwise vigorous cells. It is characterized by a large amount of deeply staining chromatic material, homogeneous and closely massed, having

no definite form but with a more or less regular outline. This mass evidently represents the nucleus, which has become filled with a diffused, swollen mass of chromatin. Its cell membrane has broken down, allowing the cytoplasm to flow out into the surrounding intercellular spaces. Notice the regular form of the chromatin nucleoli in this group of cells.

FIGURE 6. This group of eleven cells was drawn from the same cell nest as the previous group. Cell *A* is a large cell, .0093 mm. in its greatest diameter. In the cytoplasm about the nucleus are four vesicles, similar to those described under figures 2 and 4. The chromatin of its nucleus is of regular outline but is unusual on account of its size, being a trifle over one-half the diameter of the nucleus itself, at least twice the amount found in the ordinary resting cell. I think it is a cell just beginning the degeneration process shown by the swelling of its chromatin. Cell *C* is undoubtedly in the process of degeneration, for its cell membrane has disappeared, allowing the cytoplasm to flow out uncontrolled. It is a cell almost as large as cell *A*. Its chromatin, which exists as a deeply staining mass somewhat irregular in outline, was larger in diameter than that of cell *A*. The nuclear wall could not be made out. Cell *B* is one farther along in the process of breaking up than cell *C*, for most of its cytoplasm has gone. The diameter of its chromatic element is not so much larger than that of cell *C* but it is more condensed and of more regular outline. These three cells, I think, are respectively in advancing stages of disintegration. Cell *D* is a cell dividing indirectly, having a chromatic mass larger than that of cell *C* but of a looser arrangement, indicating individual chromosomes. It also maintains its cell borders perfectly.

FIGURE 7. This group of eleven cells was drawn from cell nest 6 in the region of a necrotic area. Cells *A* and *B* are in the fifth and sixth tiers respectively from the free edge of the tumor on the peripheral side of the group. I will use this group and the one following it, figure 8, to show changes in the cells passing inward from the free edge of the cell nest to a necrotic area. On the side peripheral to cell *A* there is a small necrotic spot two or three cells in diameter, which accounts for cells *A* and *B* on that side of the group. Cell *A* is a good specimen of such cells as *C* and *B* of figure 6 or cell *E* of figure 5. It shows the swelling of the chromatin and the disin-

tegration of the cytoplasm well. While there has been a swelling of the chromatin in each of these cells there has been an equally great shrinkage of the nucleus, so that at this stage there has been a real loss of material to the nucleus. Cell *B* is peculiar in that its nucleus, while practically the same size as normal, stains very dark; not solid, as in *A*, but granular. There is also a faint indication of its chromatin nucleolus remaining, not indicating so much that it has become diffused through the karyoplasm as that the karyoplasm itself assumes a new staining power. The cell wall of cell *B* is broken down in one place, showing signs of disintegration. Cell *C*, a directly dividing cell, is .0093 mm. in its greatest diameter, while cell *D* is just a trifle less than .0125 mm. The average diameter of the resting cells of this group is near .007 mm. So I think that a cell preparing for direct division increases in size to a marked extent.

FIGURE 8. This group of sixteen cells is taken from cell nest 6, the same nest that group 7 is taken from, and drawn from a zone of cells to the inner side of group 7, that is, between group 7 and a necrotic core which lies in the central part of the nest. These two figures show well the transition from the more peripheral nondegenerating cells to the entirely necrotic mass of cell debris which fills the central portion of so many of the cell nests. No dividing cells were shown. Cell *A*, the most peripheral of the group, is the only one showing a chromatin nucleolus. It is an ordinary resting cell. In cells *B* and *B'*, a type of many cells found at this zone about the necrotic area, and for that reason described as characteristic, no chromatin nucleolus could be made out through the depth of the cell, but in each of these the chromatin was scattered through the karyoplasm in the form of a loose network, staining lightly. The cell and nuclear membranes were preserved perfectly. Passing inward toward the center of the nest is a field of cells which I have called "chromatic cells," and which are perfectly characteristic of a zone of cells that immediately surrounds the necrotic core of the cell nest. They are a transition stage between the normal cells and the wholly degenerated ones. They resemble closely the disintegrating cells described in figures 5, 6 and 7, but seem to have another history of formation. As in the other cells, the nucleus is filled with a homogeneous chromatic mass, the outer layers staining darker

than those farther toward the center. In this field the stain was not as intense as that in the cells of the previous figures but was of a grayish shade, and passing toward the necrotic area the light in the gray increases until in cells *D* and *D'* there is just enough black remaining to outline the nucleus well. From this stage it gradually fades out until the cells are entirely necrotic and do not stain at all. The cell membrane in this group could not be decided upon satisfactorily. In cells *A*, *B* and *B'* it was perfectly intact, while in cells *E* it seemed to be entirely gone. In other cells, as *C*, *C'*, the cell outline was definitely preserved, while yet again in cells *D* and *D'* the cell boundary was irregular and indefinite. But I am safe in saying that when the cell has so far degenerated that the color has disappeared from the nucleus the cell membrane has also disappeared and disintegration immediately follows. But in many of these cells the cell membrane has at least partially gone while the chromatin stains deeply. The nucleus of cell *B'* is 0.004 mm. in diameter while the chromatin mass of cell *C* is near 0.003 mm. in diameter, showing an appreciable shrinkage of this element in the degeneration process.

The Mitotic Divisions Occur in General Upon the Periphery of the Alveolus, the Amitotic Nearer the Center.

I will use camera lucida drawings, together with tables, to prove this. I counted the number of cells, the number of direct divisions and the number of indirect, and also counted the tier of cells from the periphery of the cell nest in which each division occurred. I hoped by this method to arrive at some conclusion as to the rapidity of growth of the tumor, in order to compare it later with the growth of the adult and embryonic mammary tissue. I also wished to decide as to the relative frequency in occurrence of the direct and indirect divisions and to note, if possible, whether there was any comparison between the relative proportions of these and the sort of nests in which they occurred. I also wished to decide if there was a well-founded relation between either of these methods of cell division and the necrotic areas indicating that one or the other was related to degeneration, or that they occurred equally in the same region showing that they were equally vital methods of cell growth.

Cell Nest 2 was the non-necrotic limb of a necrotic body. It was counted up to the beginning of the necrotic area. I can-

not give the tiers of cells in which the various divisions occurred in this nest.

No. of cells.	*Direct divisions.*	*Indirect divisions.*
563	9	16

Percentage	of cells in divisions	4.42
"	of cells in direct divisions	1.50
"	of cells in indirect divisions	2.90
"	of divisions which are direct	36
"	of divisions which are indirect	64

CELL NEST 3. This nest apparently is a small nest in diameter but it has four "chromatic cells," which indicates that it is somewhat degenerating.

Tier	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*Av.*	*Total*
Direct D.	.	.	.	2	1	.	.	.	2	..	..	6.2	5
Indirect D.	3	1	.	1	1	.	.	.	.	..	..	2.3	6

No. of cells.	*Direct divisions.*	*Indirect divisions.*
253	5	6

Percentage	of cells in divisions	4.34
"	of cells in direct divisions	1.90
"	of cells in indirect divisions	2.40
"	of divisions which are direct	45.40
"	of divisions which are indirect	54.50

CELL NEST 4. This was a nest with a necrotic center .125 mm. to .1125 mm. in diameter, while around this on the edge of the nest was a zone of cells varying in width from .05 mm. to .037 mm. which was undegenerate and which constituted the number of cells counted, so that the total number of cells counted does not show the real size of the nest in point of number of cells, one-half of the area of the nest being degenerate.

Tier	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*Av.*	*Total*
Direct D.	1	.	3	1	2	1	1	1	.	1	1	5.5	12
Indirect D.	.	1	2	1	1	.	1	1	.	1	.	5.2	8

No. of cells.	*Direct divisions.*	*Indirect divisions.*
515	12	8

Percentage	of cells in divisions	3.88
"	of cells in direct divisions	2.30
"	of cells in indirect divisions	1.50
"	of divisions which are direct	60
"	of divisions which are indirect	40

CELL NEST 5. This was one of the smaller sized nests and was quite vigorous, showing no degenerating cells at all.

Tier	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*Av.*	*Total*
Direct D.	.	.	.	.	.	.	.	.	.	..	..	00	00
Indirect D.	3	.	.	1	.	.	.	.	.	..	..	1.7	4

No. of cells.	*Direct divisions.*	*Indirect divisions.*
106	00	4

Percentage of cells in divisions	3.77
" of cells in direct divisions	0
" of cells in indirect divisions	3.77
" of divisions which are direct	0
" of divisions which are indirect	100

CELL NEST 6. This was a cross section of a large alveolus, being .2875 mm. in length and .1758 mm. in width. It was regularly oval in outline. There were two degenerate spots in it at opposite sides, the size of six to eight cells. With these exceptions the cells were all counted.

Tier	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*Av.*	*Total*
Direct D.	.	1	4	1	.	3	.	2	1	..	..	5.0	12
Indirect D.	.	1	1	.	.	.	.	2	.	..	..	5.2	4

No. of cells.	*Direct divisions.*	*Indirect divisions.*
676	12	4

Percentage of cells in divisions	2.36
" of cells in direct divisions	1.70
" of cells in indirect divisions	0.60
" of divisions which are direct	75
" of divisions which are indirect	25

CELL NEST 7. This was a small, oval, non-necrotic nest.

Tier	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*Av.*	*Total*
Direct D.	.	.	.	.	.	.	.	.	.	..	..	00	00
Indirect D.	1	4	.	.	.	.	.	.	.	..	..	1.8	5

No. of cells.	*Direct divisions.*	*Indirect divisions.*
123	00	5

Percentage of cells in divisions	4.06
" of cells in direct divisions	0
" of cells in indirect divisions	4.06
" of divisions which are direct	0
" of divisions which are indirect	100

CELL NEST 9. This was a large necrotic nest, .2125 mm. in diameter, with a necrotic center .1125 mm. in diameter, which had a border of vigorous cells .05 mm. wide.

Tier	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*Av.*	*Total*
Direct D.	1	.	4	.	1	1	.	.	.	1	..	4.2	8
Indirect D.	1	.	1	.	.	.	.	.	.	..	..	2.0	2

No. of cells.	*Direct divisions.*	*Indirect divisions.*
299	8	2

Percentage of cells in divisions	3.34
" of cells in direct divisions	2.60
" of cells in indirect divisions	0.70
" of divisions which are direct	80
" of divisions which are indirect	20

In the following cell nests I have taken the serial sections of the same nest and counted them in the same way as I did the single sections of the previously counted nests. I hope in this way to in a manner corroborate my results and also to study the history of the cell nest farther.

CELL NEST 10. This nest was .4125 mm. in length, .0875 mm. in width. It was a nest that showed occasional spots of six to eight cells that had broken down, an average of two of these spots in each nest. The direct divisions occurred largely in the upper, wider end, while the indirect occurred largely in the lower, narrower end, but I have not illustrated this by sketches. It was therefore taken as a nest, non-necrotic, but near the stage when it would rapidly have become so, indicated by the degenerate spots.

CELL NEST 10, A.

Tier.	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*Av.*	*D.D.*	*I.D.*	*Cells.*
Direct D.	.	.	1	2	1	2	1	.	.	1	..	5.6	8	..	...
Indirect D.	1	6	2	3	.	2	.	.	.	..	..	3.0	..	14	532

CELL NEST 10, B.

Direct D.	1	1	2	5	1	2	.	.	.	..	..	4.2	12	..	...
Indirect D.	2	3	3	3	.	.	.	.	.	..	..	2.6	..	11	600

CELL NEST 10, C.

Direct D.	.	3	1	.	.	3	.	.	.	..	..	3.8	7	..	...
Indirect D.	2	2	.	3	2	.	.	.	.	..	..	3.1	..	9	557

CELL NEST 10, D.

Direct D.	.	1	1	3	2	2	1	.	.	..	..	4.6	10	..	...
Indirect D.	2	3	2	2	.	.	.	.	.	..	..	2.4	..	9	655

CELL NEST 10, E.

Direct D.	.	.	3	2	2	1	1	.	.	..	..	4.4	9	..	...
Indirect D.	1	1	2	1	1	1	.	.	.	..	..	3.4	..	7	654

CELL NEST 10, F.

Direct D.	.	.	4	3	2	2	.	.	.	..	..	4.1	11	..	...
Indirect D.	2	2	3	4	.	2	1	.	.	..	..	3.5	..	14	650

CELL NEST 10, G.

Direct D.	.	.	1	.	.	4	2	.	.	..	..	5.8	7	..	...
Indirect D.	3	7	2	1	.	1	1	.	.	..	..	2.6	..	15	564

No. of cells.	*Direct divisions.*	*Indirect divisions.*
4212	64	79

Percentage of cells in divisions 3.39
" of cells in direct divisions 1.50
" of cells in indirect divisions 1.80
" of divisions which are direct 44
" of divisions which are indirect 56

The direct divisions averaged 1.68 rows nearer the center than the indirect divisions.

CELL NEST 11. This nest was probably one-half of a half necrotic alveolus. A sketch of it in cell nest 11 *A* is given in figure 9. The cross-hatched area shows that part of the nest that is necrotic, while the white border about it is the area of the cells counted.

CELL NEST 11, A.

Tier.	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*Av.*	*D.D.*	*I.D.*	*Cells.*
Direct D.	.	2	.	1	.	.	.	.	.	..	..	2.6	3	..	...
Indirect D.	.	1	.	2	.	.	.	.	.	..	..	3.3	..	3	334

CELL NEST 11, B.

Direct D.	.	3	.	1	.	3	.	1	.	1	..	5.1	9	..	...
Indirect D.	2	.	1	.	.	.	.	.	.	..	..	1.6	..	3	305

CELL NEST 11, C.

Direct D.	.	.	.	1	1	3	.	1	.	..	..	5.8	6	..	...
Indirect D.	1	4	.	1	.	1	.	.	.	..	..	2.7	..	7	279

CELL NEST 11, D.

Direct D.	1	.	2	1	1	2	1	.	.	..	..	4.3	8	..	...
Indirect D.	1	2	1	.	.	.	.	.	.	..	..	2.0	..	4	296

CELL NEST 11, E.

Direct D.	.	1	1	.	2	.	.	.	1	.	..	4.8	5	..	...
Indirect D.	1	1	.	1	1	.	.	.	.	..	..	3.0	..	4	279

CELL NEST 11, F.

Direct D.	.	.	1	.	1	.	1	.	.	1	..	6.2	4	..	...
Indirect D.	2	.	2	.	.	3	.	.	.	..	..	3.7	..	7	271

CELL NEST 11, G.

Direct D.	.	2	1	1	3	.	.	1	1	1	..	5.3	10	..	...
Indirect D.	3	1	.	.	1	.	.	.	..	..	..	2.0	..	5	404

CELL NEST 11, H.

Direct D.	.	2	2	1	.	.	1	.	1	..	..	4.2	7	..	...
Indirect D.	.	3	1	1	1	.	.	.	.	..	..	3.0	..	6	391

CELL NEST 11, I.

Direct D.	.	.	2	.	2	2	.	.	1	1	..	5.8	8	..	...
Indirect D.	.	3	1	2	2	.	1	.	.	..	..	3.6	..	9	393

CELL NEST 11, J.

Direct D.	.	1	.	.	.	.	2	.	.	..	..	5.3	3	..	...
Indirect D.	1	.	1	3	1	.	.	.	.	..	..	3.5	..	6	306

No. of cells.	*Direct divisions.*	*Indirect divisions.*
3258	63	54

Percentage of cells in divisions	3.59
" of cells in direct divisions	1.90
" of cells in indirect divisions	1.60
" of divisions which are direct	53.80
" of divisions which are indirect	46.10

The direct divisions in this nest averaged 2.1 rows farther away from the free edge of the nest than the indirect, or 2.1 rows nearer the center of the nest.

CELL NEST 8. The work upon this nest was done with greater care than that upon the previous nests. I first made a camera lucida sketch of the nest in outline, then went over the nest carefully with the high power 8x compensating ocular and marked each division I found upon the outlined sketch. I then used an 8x Huyghenian ocular, upon the diaphragm of which I had placed a micromillimeter scale, and went over the field again, carefully counting the cells by the aid of the micrometer scale, and verified the previous locations of the dividing cells and located any new ones that I may have missed in the first survey. I thus corroborated my work and made it doubly sure by locating each dividing cell upon an outline sketch. I also used these sketches in the hope that together with the tables I might farther establish the apparent relation of directly dividing cells to the degenerating areas. Where this relation has been especially noticeable I have made high-power camera lucida drawings of the particular field to show some detail about the cells I counted, simply drawing the more important structures in relation to them. Using these large drawings and marking their position upon the smaller sketches one cannot avoid showing in the same way the slide does the real relation of the various dividing cells to these necrotic areas. In these outline sketches I only outlined the nest, tracing into any irregularities among the cells along the edge, so that, unless otherwise marked, rifts in the nests will mean spaces left where the cells have apparently been pulled apart by external means. The areas that are entirely necrotic will not be distinguished from those that show definite degenerating changes; cells that are in the "chromatic cell" stage I have counted as necrotic. These areas are shown by cross-hatched work. The indirect divisions are shown by a dot or "×" while the direct divisions are shown by a circle. Starting with figure 10 the sections are in serial arrangement. This cell nest 8 is only a portion of the entire alveolus, showing at the right side of the sketch just the end of a necrotic plug, and leaving the greater part shown in the vigorous condition. I used this nest to contrast, if possible, the two divisions in these two areas.

FIG. 10. CELL NEST 8, A.

Tier.	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*Av.*	*D.D.*	*I.D.*	*Cells.*
Direct D.	.	1	.	1	3	.	.	2	1	..	..	5.4	8	..	...
Indirect D.	2	4	4	1	2	2	.	.	.	..	..	3.2	..	15	534

FIG. 11. CELL NEST 8, B.

Direct D.	.	1	2	3	2	.	1	.	.	..	..	4.1	9	..	...
Indirect D.	1	4	4	3	1	2	.	.	.	..	..	3.3	..	15	511

FIG. 12. CELL NEST 8, C'.

Direct D.	.	1	1	1	5	1	3	2	1	..	..	5.7	15	..	...
Indirect D.	7	3	3	3	2	1	4	1	.	..	..	3.5	..	24	554

FIG. 14. CELL NEST 8, C.

Direct D.	.	1	2	2	2	2	.	1	.	..	..	4.6	10	..	...
Indirect D.	2	2	2	5	2	1	1	1	.	..	..	4.0	..	16	555

CELL NEST 8, D.

Direct D.	1	2	.	1	2	3	2	.	.	..	..	5.1	11	..	...
Indirect D.	3	1	3	3	4	1	1	.	.	..	..	3.6	..	16	626

SUBNEST 8, D.

Direct D.	.	.	.	1	1	.	.	.	.	..	..	4.5	2	..	...
Indirect D.	3	5	1	.	.	.	.	.	.	..	..	1.7	..	9	172

CELL NEST 8, E.

Direct D.	.	1	2	2	3	2	.	.	.	..	1	4.9	11	..	...
Indirect D.	4	3	7	4	4	.	.	.	.	..	..	3.0	..	22	869

CELL NEST 8, F.

Direct D.	.	.	3	2	6	2	3	.	.	..	..	5.0	16	..	...
Indirect D.	3	5	3	2	2	2	.	.	.	..	..	3.0	..	17	745

FIG. 16. CELL NEST 8, G.

Direct D.	.	2	3	1	3	4	1	1	.	..	..	4.7	15	..	...
Indirect D.	2	6	2	1	.	1	1	.	.	..	..	2.8	..	13	916

FIG. 18. CELL NEST 8, H.

Direct D.	1	2	3	1	.	3	3	.	.	..	..	4.3	13	..	...
Indirect D.	3	1	7	1	.	.	2	.	1	..	..	3.5	..	15	907

FIG. 19. CELL NEST 8, I.

Direct D.	1	.	2	1	2	.	1	3	.	..	1	5.7	11	..	...
Indirect D.	1	1	7	2	4	1	1	.	.	..	..	3.8	..	17	912

No. of cells.	*Direct divisions.*	*Indirect divisions.*
7301	121	179

Percentage	of cells in division	4.01
"	of cells in direct division	1.60
"	of cells in indirect division	2.40
"	of divisions which are direct	40.33
"	of divisions which are indirect	59.66

The direct divisions of this nest averaged 1.78 rows nearer the center than the indirect.

DESCRIPTION OF OUTLINE SKETCHES AND DRAWINGS.

CELL NEST 8, "A" (fig. 10). The amount of necrotic material in this figure is greater than in the following ones, the necrotic plug showing less and less in each consecutive section. There are no divisions shown in this figure near the necrotic plug, but the nearest two are direct. The greater part of the dividing cells are in the limb of the nest.

CELL NEST 8, "B" (fig. 11). The number of divisions present in the distal part of the limb is noticeable. There are three direct divisions immediately adjacent to the necrotic plug and one nearer than the nearest indirect division.

CELL NEST 8, "C" (fig. 12). In this nest there was an unusually large number of dividing cells. At the point of the arrow in figure 12 is the center of the group of cells drawn on a large scale in figure 13. About the arrow point are the four direct divisions and the two indirect divisions shown in the enlarged group. This group of nineteen cells includes a larger area under the lens than any previously drawn. I drew all the cells of importance under the field of the camera lucida, including a field .05 mm. in diameter, while the previous groups drawn have included an area only .0375 mm. in diameter. It is located on the edge of the necrotic area and includes the whole width of cells lying between this area and the free edge of the cell nest, cell *F* being in the first row of the nest, while cell *B* is among the necrotic cells. The direct divisions of this section are deep in the center of the nest, while the indirect are largely very close to the edge, seven cells out of twenty-five dividing indirectly lying in the first row and no directly dividing cells nearer than the second row, and then only one. A general survey of these sketches together with the tables strongly indicates that the cells on the edge of the tumor increase almost, if not wholly, by indirect divisions, while those deeper away from the edge increase largely by direct divisions but occasionally by indirect. I want to emphasize by the large drawings the intimate association of the directly dividing cells with the degenerate ones. Cells *H*, *H'*, *H''* are the "chromatic cells" that I have described previously under figure 13. Surrounding these cells closely are cells *A*, *B*, *C*, *D* and *E*, plainly in the process of direct division. Cell *B* is peculiar in that it shows no chromatin nucleolus in either segment of the nucleus. This same occurrence was noticed in numbers

of resting cells, *B*, *B'*, figure 8, etc., just outside the zone of "chromatic cells" *H*, *H'*, *H''*. Its chromatin had wholly or largely disappeared, and if any remained it was loosely diffused through the karyoplasm. Cell *E* is another cell showing no chromatin in any of the segments of its nucleus. It evidently was in decay for it was very translucent. The fragments of its nucleus showed no chromatin at all. It may be that a cell such as *B*, which has vigor enough to cause its nucleus to segment but not to cytoplasm, gradually loses its staining ability and its definiteness of outline and later becomes merely a mass of debris through a stage represented in cell *E*. Cells *F* and *G* are two cells in the indirect division process. The directly dividing cells are much larger than the resting cells or those dividing indirectly. Cell *A* is .0093 mm. in its greatest diameter, cell *B* is .007 mm., cell *C* is .0093 mm., cell *G* is .0062 mm., cell *F* is .0062 mm., cell *E* is .0093 mm., cell *D* is .0085 mm., while cell *I*, an average resting cell, is .0062 mm. in its greatest diameter. This cell shows the breaking up of the chromatin and its diffusion through the karyoplasm, possibly preceding cells *H*, *H'*, *H''*. Its nucleus is .005 mm. in diameter, while the diameter of the "chromatic mass" in cells *H*, *H'*, *H''* is .0031 mm., showing evidently a shrinkage in the latter.

FIGURE 14. In this section two indirect divisions are shown located on the very edge of the necrotic plug, and two more near. There are also close to the edge of the nest three direct divisions, still showing a greater proportion of direct divisions near the necrotic area than indirect, when compared with the total number of divisions of each in the section. But this shows that indirect division may occur on the very edge of a degenerating area.

At the lower extremity of the limb of the regular section following is found a circular group of cells, which in the next section shows a connection with the larger body. This is the first appearance of this group, but in the previous sections there is a circular space indicating where it has been but from which it has in some way been lost. There is a cleft entirely through the regular section, but this was where the cells had pulled apart and not where they had degenerated. The direct divisions are intimately associated with the necrotic spots. In the middle segment there are two indirect divisions on the edge

of the necrotic area, but usually they are near the free edge of the nest.

In the succeeding section the subnest has joined the limb of the main body and it was all counted together. There are three necrotic areas in this section. In the lower one the two directly dividing cells of that region are on the edge of the degenerated area.

A later section shows the nest in two segments, broken apart from each other. A pluripolar cell division shows at one place.

The group of cells drawn under high power in figure 15 is from this section. Figure 15 shows a group of five directly and one indirectly dividing cells. In this drawing and those made hereafter I shall indicate the segments of the nucleus as they appear through the depth of the cell, demonstrating their overlapping. The border line used was drawn at the edge of the cells which presented no marked degeneration changes, but it is not a hard, fast line, a few vigorous cells jutting out farther than the line and a few chromatic cells being behind it. Cells *A, B, C, D* and *E* show well-marked direct divisions. Cell *A* is on the necrotic border. Cell *B,* a very small cell for a directly dividing one, is a half cell width from the necrotic border. Cell *C* is a trifle closer than *B* to the border. Cell *D* is in the third row from the border. It is peculiar in that its chromatin nucleoli in each segment are not staining deeply and the chromatin is diffused through the karyoplasm. Cell *E* is in the fourth row from the necrotic edge. Cell *F* is an indirectly dividing cell and is in the same row of cells as cells *C* and *B.* Cells *G* and *G'* are the "chromatic cells" described under figure 13. They mark the edge of the necrotic plug and are in various stages of degeneration, as indicated by the disintegration of their cytoplasm and the loss of the staining power of their chromatin. This sketch together with the smaller one shows well this collecting of large numbers of the directly dividing cells about these necrotic areas. There are mitotic cells here, but not in such proportion in comparison to their total number as the amitotic.

FIGURE 16. In this section there are more direct divisions than indirect. There is a noticeable change in the number of indirect divisions in the lower part of the nest, in what was termed a "subnest" in a preceding section. In one section there are nine indirect divisions, two direct. In the next there

are eight indirect divisions, two direct. In the next there are five indirect divisions, three direct. In a later (fig. 16), there are five indirect divisions, four direct. In another (fig. 19), there are four indirect divisions, three direct. This shows consistently in this case that as the center of the nest is approached in the sections the number of direct divisions increase in proportion while the indirect decrease in proportion. In the region of the arrow point of figure 16 an enlarged drawing was made (figure 17). I used this to show more detail about the cells shown in figure 16. Cell *A* was on the edge of the necrotic area. Its chromatin was small in amount and gathered in two clumps in one segment and three in the other. Cell *C* is an ordinary dividing cell. Cell *D* is in the second row from the necrotic edge. The cross-hatched work shows the necrotic area, this area being entirely necrotic and showing no transition cells along the edge.

FIGURE 18. There are but two dividing cells in the upper segment of this nest, while in an earlier there are fourteen, the number decreasing with each section until this one. They have increased steadily in the middle part of the limb, however, from eleven to nearly twenty in the section shown in figure 18. This carefully worked out will demonstrate that at the same time the cells in one part of the nest are rapidly dividing those in another part are not dividing at all. There is either an unequal stimulus to growth in the nest or an unequal negative cause.

FIGURE 19. This nest is the same one as that in the preceding figure, but in this section the two parts have been separated. At the point of the arrow is the location of figure 20, and here are shown the three dividing cells drawn in figure 20. In figure 20, cell *A* is plainly among the degenerating cells in the necrotic plug, and is surrounded by "chromatic cells," no vigorous cell being near. It is a large clear cell with a bisegmented nucleus and showing but very little chromatin. Its appearance indicates that it is a degenerating cell. Cell *B* lies just outside the border of vigorous cells. Its nucleus is bisegmented and it stains much deeper than the cytoplasm. The chromatin nucleoli stain very faintly. Cell *C* is a large clear cell lying in the third row of cells from the necrotic edge. It stains lighter than the other cells about it and shows a small amount of chromatin and that stained faintly. Cells *A* and *B*

are noticeable because they have broken off from the vigorous growth and lie among cells that are distinctly necrotic.

Out of the 294 direct divisions in all the nests only 7 occurred in the first row on the edge of the nest.

Out of the 357 indirectly dividing cells 63 occurred in the first row on the edge of the nest.

The direct divisions in all the nests averaged 1.68 rows nearer the center than the indirect divisions.

CELL NEST 12. This nest was sketched and counted in forty-two serial sections. It began as three or four groups of cells which were the cross sections of limbs which later fused into a common trunk. It was non-necrotic throughout.

CELL NEST 12, I.

Tier	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*Av.*	*D.D.*	*I.D.*	*Cells*
Direct D.	.	.	1	.	.	.	.	3.0	1	..	...
Indirect D.	2	1	4	.	.	.	.	2.2	.	7	166

CELL NEST 12, II.

Direct D.	.	.	.	.	.	.	.	..	.	..	...
Indirect D.	.	4	.	1	1	.	.	2.8	.	6	140

CELL NEST 12, III.

Direct D.	.	2	.	.	.	.	.	2.0	2	..	...
Indirect D.	.	1	.	.	.	.	.	2.0	.	1	78

CELL NEST 12, IV.

Direct D.	.	.	1	.	.	.	.	3.0	1	..	...
Indirect D.	2	.	.	1	.	.	.	2.0	.	3	77

CELL NEST 12, V.

Direct D.	.	.	.	.	.	.	.	..	.	..	...
Indirect D.	2	3	1	.	.	.	.	1.8	.	6	180

CELL NEST 12, VI.

Direct D.	.	.	1	.	.	.	.	3.0	1	..	...
Indirect D.	.	1	1	.	.	.	.	2.5	.	2	81

CELL NEST 12, VII.

Direct D.	.	1	1	.	.	.	.	2.5	2	..	...
Indirect D.	.	.	2	1	.	.	.	2.3	.	4	85

CELL NEST 12, VIII.

Direct D.	.	.	.	.	.	.	.	..	.	..	...
Indirect D.	1	3	.	3	.	.	.	2.7	.	7	83

CELL NEST 12, IX.

Direct D.	.	.	1	1	.	.	.	3.5	2	..	...
Indirect D.	1	1	1	.	.	.	.	2.0	.	3	86

CELL NEST 12, X.

Direct D.	.	.	.	2	.	.	.	4.0	2	..	...
Indirect D.	2	2	.	.	.	.	.	1.4	.	4	169

CELL NEST 12, XI.

| | | |

CELL NEST 12, XXVI.

| | | |

CELL NEST 12, XLI.

Direct D.	.	2	.	.	.	.	.	2.0	2	..	...
Indirect D.	2	.	.	.	.	.	3	4.6	.	5	371

CELL NEST 12, XLII.

Direct D.	.	1	1	.	.	2	.	4.2	4	..	...
Indirect D.	4	2	3	.	.	1	.	2.3	.	10	421

No. of cells.	*Direct divisions.*	*Indirect divisions.*
12,228	81	285

Percentage of cells in divisions	2.99
" of cells in direct divisions	.6
" of cells in indirect divisions	2.3
" of divisions which are direct	22.1
" of divisions which are indirect	77.9

Table of all the nests counted, showing percentage of cells in division, percentage of cells in direct division, and percentage of cells in indirect division:

Cell nest.	*Percentage of cells in division.*	*Percentage of cells in direct division.*	*Percentage of cells in indirect division.*
2	4.42	1.5	2.9
3	4.34	1.9	2.4
4	3.88	2.3	1.5
5	3.77	0.0	3.77
6	2.36	1.7	.6
7	4.06	0.0	4.06
8	4.01	1.6	2.4
9	3.34	2.6	.7
10	3.39	1.5	1.8
11	3.59	1.9	1.6
12	2.99	.6	2.3

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CONCLUSIONS.

1. The percentage of cells in division shows no relation to the percentage of amitoses or mitoses.

2. The percentage of mitoses is high in small non-necrotic nests.

3. The percentage of amitoses is high in large necrotic nests, indicating that it is the division associated with degeneration.

4. Amitosis is the form of division occurring in the necrotic area.

5. The rate of division in the various nests is inconstant.

6. The directly dividing cells lie nearer the center of the cell

nests and thus nearer the necrotic areas than the indirectly dividing cells.

7. The indirectly dividing cells lie nearer the periphery of the nest than the directly dividing cells.

8. From these observations it may therefore reasonably be concluded that amitosis represents the final effort of the cell at reproduction, and that shortly after its occurrence degeneration ensues.

9. Amitosis, mitosis and disintegration comprise the cell changes in the alveoli.

10. Cells in direct and indirect division are larger than the resting cells.

11. In one stage of degeneration of the cells the chromatin becomes diffused through the karyoplasm and so swells that it fills the nucleus as a deeply staining, homogeneous mass. In another process of degeneration the chromatin loses its staining power and the cell becomes clear.

12. There is a shrinkage of the nuclear bulk in degeneration.

13. A border of chromatic cells lies between the vigorous cells and the cell debris of the necrotic area and marks the limit of degeneration; such cells are themselves degeneration results.

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